7.2 FECAL INDICATOR VIRUSES

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[1]See Appendix A7-A, Table 3, for parameter codes used in the National Water Information System (NWIS) of the U.S. Geological Survey for somatic and F-specific coliphages.

7.2 FECAL INDICATOR VIRUSES

More than 100 types of human pathogenic viruses may be present in fecal-contaminated waters, but only a small number of them can be detected by currently available methods (Havelaar and others, 1993). Coliphages are used as indicators of fecal contamination and of the microbiological quality of the water.[2] Coliphages are viruses that infect and replicate in coliform bacteria and are not pathogenic to humans; coliphages have been suggested as potential indicators of enteric viruses because of their similar structure, transport, and persistence in the environment (Gerba, 1987).

Two main groups of coliphages are used as viral indicators:

Coliphages: Viruses that infect and replicate in coliform bacteria. Coliphages are used as indicators of fecal contamination in water.

- Somatic coliphages infect coliform bacteria by attaching to the outer cell membrane or cell wall. They are widely distributed in both fecal-contaminated and uncontaminated waters.
- F-specific coliphages attach only to hairlike projections (called F pili) of coliform bacteria that carry an extrachromosomal genetic element called the F plasmid; F pili are produced only by bacteria grown at higher temperatures. F-specific coliphages presumably come from warm-blooded animals or sewage (Handzel and others, 1993).

[2]The term "fecal indicator viruses" is used synonymously with coliphages in this report, as coliphage analysis currently is the only standard viral method used by the U.S. Geological Survey for indicating fecal contamination.

Somatic and F-specific coliphages are found in high numbers in sewage and are thought to be reliable indicators of sewage contamination of waters (International Association of Water Pollution Research and Control Study Group on Health Related Microbiology, 1991). Raw sewage typically contains somatic and F-specific coliphage concentrations of about 1,000 plaque-forming units per milliliter (Sobsey and others, 1995).

Two methods are commonly used to analyze samples for somatic and F-specific coliphages:

- The single-agar layer (SAL) method is recommended for use with surface-water samples. It is a quantitative, plaque assay method that can analyze sample volumes of 100 mL (milliliters).
- The two-step enrichment method is recommended for use with ground-water samples. It is a presence/absence method that can analyze sample volumes of either 100 mL, 1 L (liter), or 4 L.

Coliphage methods of analysis must be performed in the laboratory by a trained microbiologist.

The type of coliphage detected by these methods depends on the bacterial host strain used. Two host strains commonly used for the detection of somatic coliphages are *Escherichia coli* (*E. coli)* C and *E. coli* CN-13. Both hosts are equivalent in coliphage detection; however, *E. coli* CN-13 is resistant to nalidixic acid and is preferable for analyzing samples with a high background or unknown level of indigenous bacteria (Sobsey and others, 1995). Antibiotics such as nalidixic acid are used to minimize overgrowth of indigenous bacteria in environmental samples; this overgrowth may mask the detection of coliphage. Three host strains commonly used for the detection of F-specific coliphages are *E. coli* F-amp, *E. coli* C3000, and *Salmonella typhimurium* WG49. The *E. coli* F-amp strain appears to be the most reliable host for detecting only F-specific coliphages; the F-amp strain is resistant to ampicillin and streptomycin, so it is less susceptible to bacterial contamination in water samples (Sobsey and others, 1995).

SAMPLING EQUIPMENT AND EQUIPMENT STERILIZATION PROCEDURES 7.2.1

Sterile techniques must be followed and documented when collecting and processing samples for fecal indicator viruses. **The specific equipment and supplies that are needed to collect and analyze samples for fecal indicator viruses must be kept clean and sterile** (tables 7.2-1, 7.2-2). The equipment and procedures described in the following paragraphs are applicable to fecal indicator viruses and to fecal indicator bacteria (NFM 7.1). Equipment to be autoclaved must first be wrapped in aluminum foil, autoclavable bags, or kraft paper. Non-autoclavable equipment must be cleaned and, if possible, sterilized and then similarly wrapped for storage and transport.

- Sterilize and store the equipment in a clean area.
- Resterilize equipment if foil, bag, or kraft paper is torn.

Add sodium thiosulfate ($Na_2S_2O_3$) to sample bottles before sterilization if the water to be collected is suspected to contain residual chlorine or other halogens. $Na_2S_2O_3$ may also be added to the sample bottle immediately after sample collection. Residual chlorine commonly is found in treated (disinfected) potable water (for example, public water systems), and in sources such as wastewater effluents or mixing zones directly downstream from wastewater-treatment plants. Most taps or wells (for example, small private water systems) do not contain residual chlorine.

Autoclaving is the preferred method for sterilizing equipment.

Table 7.2-1. Equipment cleaning and sterilization procedures

[DIW, distilled or deionized water; mL, milliliter; $Na_2S_2O_3$, sodium thiosulfate; °C, degrees Celsius; mg/L, milligrams per liter]

Equipment	Cleaning and sterilization procedures
All equipment (this includes water-level tape measure, all sample-collection and sample-processing equipment used in the field and laboratory)	Wash equipment thoroughly with a dilute nonphosphate, laboratory-grade detergent. Rinse three times with hot tap water. Rinse again three to five times with DIW. Wipe down the wetted portion of water-level tapes with disinfectant (0.005-percent bleach solution or methyl or ethyl alcohol) and rinse thoroughly with DIW.
Autoclavable glass, plastic, and Teflon bottles	If sample will contain residual chlorine or other halogens, add 0.5 mL of a 10-percent $Na_2S_2O_3$ solution per liter of sample to the sample bottles. Wrap all autoclavable equipment in aluminum foil, kraft paper, or place into autoclavable bags.[1] Autoclave at 121°C for 15 minutes.
Portable submersible pumps and pump tubing	**Autoclavable equipment** (preferred): Wrap components in aluminum foil, kraft paper, or place into autoclavable bags. Autoclave at 121°C for 15 minutes. **Non-autoclavable equipment: (1)** Submerge sampling system into a 50-mg/L (0.005 percent) sodium hypochlorite solution prepared from household laundry bleach. **(2)** Circulate solution through pump and tubing for 30 minutes. **(3)** Follow step (2) by thoroughly rinsing, inside and out, with 0.5 mL of a 10-percent sterile $Na_2S_2O_3$ solution per liter of water and circulate solution for 5 minutes; **(4)** pump $Na_2S_2O_3$, discarding this waste appropriately; pump sterile DIW through the pump, followed by pumping three tubing volumes of well water to waste (discard appropriately) before collecting the sample. CAUTION: Prolonged or repeated use of a hypochlorite solution on interior or exterior metallic surfaces of a pump can cause corrosion or other damage to the pump and compromise the quality of samples collected for trace-element or organic-compound analysis.

[1]Equipment to be wrapped in aluminum foil, kraft paper, or placed into autoclavable bags includes, for example, bottles, tubing, flasks, bailers, pump components. The $Na_2S_2O_3$ solution also is autoclaved.

To prepare for collecting a halogenated sample:

1. Prepare a 10-percent solution of $Na_2S_2O_3$ as follows:
   a. In a volumetric flask, dissolve 100 g of $Na_2S_2O_3$ into 500 mL of deionized or distilled water (DIW).
   b. Stir until dissolved.
   c. Fill the flask to 1,000 mL (Bordner and Winter, 1978, p. 6; American Public Health Association and others, 1998, p. 9-19). Autoclave at 121°C for 30 minutes (U.S. Environmental Protection Agency, 1996, p. VIII-II).
   d. Store the $Na_2S_2O_3$ solution at room temperature or under refrigeration. After 6 months prepare a fresh solution.
2. Before collecting the sample, pipet into the sample bottle 0.5 mL of 10-percent $Na_2S_2O_3$ solution for every 1 L of sample. If the sterile $Na_2S_2O_3$ is used, be sure to use only sterile pipets and sterile sample bottles. If the $Na_2S_2O_3$ is not sterile, dispense the required volume of $Na_2S_2O_3$ into the sample bottle and autoclave at 121°C for 15 minutes.

$Na_2S_2O_3$ solution has a 6-month shelf life. Discard unused solution that has expired, prepare fresh solution, and label bottle with date of preparation.

Clean and sterilize sampling equipment (table 7.2.2)**.** All equipment, including tubing and containers, must be cleaned and sterilized between sites or for each sample collected at the same site at different times. Autoclaving is the preferred method of sterilization.

- Use only autoclaves that have temperature, pressure, and liquid- and dry-utensil-cycle controls. Steam sterilizers, vertical autoclaves, and pressure cookers without temperature controls are not recommended.
- Take care to ensure that materials to be autoclaved are thermally stable. Plastics (such as polycarbonate, polypropylene, polyallomer, and polymethylpentene) and Teflons and Tefzel (such as perfluoroalkyoxypolymers or PFA, ethylenetetrafluoroethylene or ETFE, fluorinated ethylene propylene or FEP, and polytetrafluoroethylene polymers or PTFE) can be autoclaved. **Note that each of these materials has different thermal characteristics and tolerances to repeated autoclaving.**
- In addition to the guidance listed above, it is necessary to:
  - Use sterilization indicator tape with each load.
  - Use commercially available biological indicators at least quarterly to test autoclave performance. Biological indicators are composed of endospores—living cells that are resistant to heat, but are destroyed by autoclaving.
  - Drain the autoclave at the end of each period of use. Clean with mild soap and water once per week during periods of daily use. Record cleaning dates in the logbook.
  - Autoclave cultures of microorganisms and all media plates for at least 30 minutes before disposal.
  - Wrap silicone tubing in kraft paper or aluminum foil before autoclaving.
  - Avoid overloading the autoclave with equipment or materials; overloading will result in incomplete sterilization.

- The 20th edition of "Standard Methods for the Examination of Water and Wastewater" (American Public Health Association and others, 1998, p. 9-2 to 9-14) contains specifications for the length of time, temperature, and pressure for autoclave sterilization of various media and materials.

Quality control in sterilization procedures are mandatory. Keep a logbook of autoclave operation. Enter into the logbook the quality-assurance and quality-control procedures used, noting the date, the test results, and the name of the autoclave operator and (or) analyst. Record the autoclave temperature, pressure, date, and time of each autoclave run. If the autoclave does not reach the specified temperature and pressure or fails a quality-control test, then the autoclave should be serviced and all materials resterilized (American Public Health Association and others, 1998, p. 9-2 to 9-14).

7.2.2 SAMPLE COLLECTION, PRESERVATION, TRANSPORT, AND HOLDING TIMES

Sterile conditions must be maintained during collection, preservation, transport, and analysis of fecal indicator virus samples. Specific procedures have been developed that must be strictly followed. These procedures vary with types of sampling equipment and sources of sample (surface water, ground water, treated water, or wastewater).

A summary of requirements for sample-collection containers and procedures for sample preservation is given in table 7.2-2.

Table 7.2-2. Summary of equipment for sample collection and procedures for sample preservation of fecal indicator viruses

[EWI, equal-width-increment; EDI, equal-discharge increment; L, liter; mL, milliliter; $Na_2S_2O_3$, sodium thiosulfate; °C, degrees Celsius]

Equipment for sample collection (All containers must be composed of sterilizable materials such as borosilicate glass, polypropylene, stainless steel, or Teflon)
To collect EWI or EDI surface-water samples: US D-95, US DH-95, or US DH-81 with sterile 1-L wide-mouth bottle, caps, and nozzles. US D-96 with sterile autoclavable bag (NFM 2.1.1).
To collect surface-water and ground-water samples using point samplers or hand-dipped method: a sterile, narrow-mouth container, 500 mL to 1 L capacity, or a sterile 3-L container if both types of coliphages are to be analyzed.
To collect pumped samples: Use sterile tubing, clean and sterile pump components (autoclaved, if possible; see text).
Procedures for sample preservation
Before sample collection, if halogen neutralization is necessary, add 0.5 mL of a 10-percent $Na_2S_2O_3$ solution per 1 L of sample. - If sterile $Na_2S_2O_3$ is used, dispense with sterile pipet into sterile bottle. - If $Na_2S_2O_3$ is not sterile, dispense with pipet into sample bottle and autoclave (table 7.2-1). Chill all samples at 1-4°C before analysis.

7.2.2.A SURFACE-WATER SAMPLE COLLECTION

The areal and temporal distribution of fecal indicator viruses in surface water can be as variable as the distribution of suspended sediment because viruses commonly are associated with solid particles. To obtain representative data for fecal indicator virus analysis, follow the same methods used to collect surface-water samples for suspended sediment analysis (Edwards and Glysson, 1999; NFM 4.1).

- **For flowing water,** use depth-and-width-integrating sampling methods[3] (NFM 4.1.1.A).
- **For still water** (lakes or reservoirs, or other surface-water conditions for which depth-and-width-integrating methods are not applicable), use the hand-dip method or a sterile point sampler (NFM 4.1.1.B).

[3]Sample-collection methods may be modified to ensure consistency with study objectives and as appropriate for site conditions. It is necessary to describe any methods modifications in a report of the results of the study.

- **For beach water,** use a hand-dip method in shallow wadable water and a sterile point sampler for deeper water. Collect samples by the hand-dip method at knee depth, a depth of approximately 15 to 30 cm (6 to 12 in.) below the water surface.
  - — Collect samples near known or suspected pollution sources, in areas of concentrated activity (for example, near lifeguard chairs), or for approximately every 500 m (every quarter mile) of beach length (U.S. Environmental Protection Agency, 2002).
  - — Position the sampler downstream from any water currents to collect the sample from the incoming flow (U.S. Environmental Protection Agency, 2002) and record sampling location. Avoid contaminating the water sample with bottom material dislodged by disturbing the bottom while sampling.
  - — A Chain of Custody (COC) record is recommended for beach sampling done in support of beach closures or posting of warnings to swimmers (U.S. Environmental Protection Agency, 2002, Appendix J).

Always wear laboratory gloves when handling sampling equipment and samples. Take care to prevent contaminated water from contacting skin, mouth, nose, or eye areas (NFM 9.7).

Depth-and-width-integrating methods

Depth-and-width-integrating sampling methods (the equal-discharge-increment (EDI) method or the equal-width-increment (EWI) method) are the standard USGS methods used when sampling flowing waters, and are recommended unless study objectives or site characteristics dictate otherwise (NFM 4.1.1.A).

1. Select the EDI or EWI method. The EDI method is preferred to the EWI method for sites where the velocity distribution across a stream section is well established or at a section where the depth varies; for example, at a gaging station (Edwards and Glysson, 1999).

2. Select the appropriate sampler and equipment. **Sampling equipment must be sterile,** including the collection bottle, nozzle, and cap (or bags for the bag sampler) (table 7.2-1).
   - For streams with depths of 5 m (16.4 ft) or less, use a US D-95, US DH-95, or a US DH-81 sampler (NFM 2.1.1).
   - For stream sections where depths exceed 5 m (16.4 ft), use the US D-96, with either autoclavable Teflon bags or autoclavable cooking bags. Thermotolerant polymers are described in more detail in section 7.2.1 under "Sampling Equipment and Equipment Sterilization Procedures."
   - For compositing subsamples, use a sterile 3-L or larger bottle.
     — For wide channels, several samples, each composed of subsamples composited into a sterile large-volume container, may be needed.
     — For narrow channels, collect subsamples at 5 to 10 or more vertical locations in the cross section without overfilling the bottle.
   - Use the proper nozzle size and transit rate for the velocity conditions in the section to ensure isokinetic collection of the sample.

Hand-dip method

If the stream depth and (or) velocity is not sufficient to use a depth-and-width-integrating method, collect a sample using a hand-dip method. Sampling still water or sampling at depth in lakes, reservoirs, estuaries, and oceans requires a sterile point sampler. Niskin, ZoBell, and Wheaton point samplers hold a sterilizable bottle or bag. Wearing laboratory gloves, collect a hand-dipped sample as follows:

1. Open a sterile, narrow-mouth borosilicate glass or plastic bottle; grasp the bottle near the base, with hand and arm on the downstream side of the bottle.
2. Without rinsing, plunge the bottle opening downward, below the water surface. Allow the bottle to fill with the opening pointed slightly upward into the current.

3. Remove the bottle with the opening pointed upward from the water surface and tightly cap it, allowing about 2.5 to 5 cm of headspace (American Public Health Association and others, 1998, p. 9-19; Bordner and Winter, 1978, p. 8). This procedure minimizes collection of surface film and prevents contact with the streambed.

Do not sample in or near an open water body without wearing a correctly fitted personal flotation device (PFD).

Quality control in surface-water sampling. Depending on the data-quality requirements of the study and site conditions, quality-control (QC) samples (field blanks, field replicates, and matrix spikes) generally constitute from 5 to 20 percent or more of the total number of samples collected over a given period of time. See "Selected Terms and Symbols" in the Conversion Factors section at the end of this chapter, which contains definitions of the quality-control terms shown below in bold type.

- **Field blanks**—Collect field blanks at a frequency of 1 in every 10 to 20 samples to document that sampling equipment has not been contaminated. Process field blanks before collecting the water sample as follows:

1. Pass sterile DIW through sterile sampling equipment and into a sterile sample container.
2. Analyze sterile DIW for fecal indicator viruses. If no viruses are observed, then the sample was collected by use of sterile procedures.

- **Field replicates**—Collect one field replicate for every 10 to 20 samples.

- **Matrix spikes**—Collect a set of matrix spike samples for each coliphage type when samples are first received from a water source. Once received from a water source, collect a set of matrix spike samples after every 20th sample from that source. The matrix spike samples are spiked with known amounts of coliphage by the analyzing laboratory.

7.2.2.B GROUND-WATER SAMPLE COLLECTION

As with surface water, most viruses in ground water are associated with solid particles. Stable values of field measurements (turbidity, temperature, dissolved-oxygen concentration, pH, and specific conductance), especially turbidity and dissolved oxygen, are important criteria for judging whether a well has been sufficiently purged for the collection of a representative ground-water sample for fecal indicator virus analysis (NFM 4.2 and 6.0.3.A). Sampling equipment that has been subjected to chlorinating and dechlorinating agents can affect the chemistry of samples collected for non-microbial analysis; therefore, collect blank samples to be analyzed for chloride, sulfate, and other constituents, as appropriate, to document that sample quality has not been compromised.

- If using the same equipment for chemical-analysis and virus-analysis samples, clean the equipment by first using standard procedures (NFM 3), followed by disinfecting and rinsing procedures described in section 7.2.1. Purge the well as described in NFM 4.2 before collecting samples.
- If different equipment will be deployed in a well for virus sampling, first check for stable turbidity and dissolved-oxygen readings to ensure collection of a representative sample.

Supply wells

If samples are to be collected from a water-supply well (see definition in NFM 4.2), select a tap (spigot) that supplies water from a service pipe connected directly to the main; do not use a tap on a pipe served by a cistern or storage tank (American Public Health Association and others, 1998, p. 9-19 to 9-20; Britton and Greeson, 1989, p. 5; Bordner and Winter, 1978, p. 5-16). Avoid sampling after downhole chlorination. Dechlorination with $Na_2S_2O_3$ is required if you cannot avoid collecting the sample before the water has passed through the treatment unit.

Do not sample from leaking taps.

To sample a supply well for fecal indicator viruses:

1. Before collecting the sample, remove screens, filters, or other devices from the tap.
2. Before sampling, swab the inside and outside rim of the tap with ethanol. Flame sterilize the tap and allow it to dry and cool. Rinse the tap with sterile DIW.
3. Collect a sample directly from the tap into a sterile bottle without splashing or allowing the sample bottle to touch the tap.
   - — Supply wells commonly are equipped with permanently installed pumps. If the well is pumped daily, then (a) purge the tap water for a minimum of 5 minutes, discarding the purged water appropriately; (b) monitor field measurements and record stabilized values (NFM 6); and (c) collect the sample directly from the tap into a sterile container (described in table 7.2-2).
   - — If the well is used infrequently, then purge the tap or well of water until a minimum of three borehole volumes are purged and stable field measurements are obtained in sequential measurements (NFM 4.2 and 6.0.3.A).

Monitoring wells

If a monitoring well does not have an in-place pump, then obtain samples by using a portable sampler, such as a submersible pump or a bailer (U.S. Environmental Protection Agency, 1982). Samplers and sample lines must be sterilized or disinfected (table 7.2-1). If disinfected, then the sampler and sample line must be dechlorinated and rinsed with sterile DIW. In either case, finish by flushing the sampler and sample line with native ground water before samples are collected into sterile bottles.

- ▶ Use autoclavable samplers, if possible. After flushing the sterilized pump lines with sample, collect the sample directly into the sterile sample bottles.
- ▶ Check data-collection objectives before using a disinfectant. Disinfectants are corrosive; they can damage the metal parts of a pump, and can render the pump inadequate for trace-element sampling and other constituent sampling.

To disinfect a pump:

1. Submerge the pump and pump tubing in a 0.005 percent (50 mg/L) sodium hypochlorite solution prepared from household laundry bleach. Most bleach is about 5 to 7 percent sodium hypochlorite (50,000 to 70,000 mg/L), but bleach in a container left open for more than 60 days may not be full strength. Prepare solutions fresh with each use, because they will diminish in concentration with time. Add 1 mL of household laundry bleach to 900 mL of water and bring to a volume of 1,000 mL for a 0.005 percent disinfectant solution (U.S. Environmental Agency, 1982, p. 253 and 1996, p. VIII-41). This concentration is sufficient for waters with a range of pH between 6 and 8 and temperatures greater than 20°C. Outside these ranges, a more concentrated disinfectant solution, up to 0.02 percent (200 mg/L), should be used (U.S. Environmental Protection Agency, 1982, p. 253).
2. Circulate the disinfectant through the pump and tubing for 30 minutes.
3. Afterwards, rinse the pump thoroughly with a sterile $Na_2S_2O_3$ solution. The $Na_2S_2O_3$ solution is prepared by adding 0.5 mL of a 10-percent sterile solution to every 1 L of sterile DIW. Recirculate for 5 minutes and rinse with sterile DIW.
4. Lower the pump carefully into the well. Pump some well water to waste to remove any residual chlorine and $Na_2S_2O_3$. Take care not to contaminate samples for chemical analysis with residual disinfectant or $Na_2S_2O_3$. **The pump must have a backflow check valve (an antibacksiphon device) to prevent residual disinfectant from flowing back into the well.**

If the pump cannot be disinfected:

1. Handle the pump and tubing carefully to avoid contamination. If the pump is a downhole dedicated pump, proceed to step 2.
2. Purge the well with the pump used for sampling to allow the pump and tubing to be thoroughly flushed with aquifer water before sampling (NFM 4.2 and 6.0.3.A).
3. Collect field blanks through the sampling equipment.
4. An alternative to sampling with the pump is to remove the pump after completion of purging and collection of other samples. Collect the coliphage sample with a sterile bailer (U.S. Environmental Protection Agency, 1982, p. 252–253). If using this method, evaluate the potential for bias from stirring up particulates during pump removal and bailing that otherwise would not be included in the sample.

Sample-preparation activities, such as purging, must be carried out in such a way as to avoid contaminating the well, the equipment, or the samples. Avoid collecting surface film from the well water in the sample and ensure that the sampler intake is within the screened interval targeted for study. Select a point-source sampler, such as a bailer with a double-check valve. Do not use a bailer unless the bailer can be sterilized. The type of well, its use, construction, composition, and condition could lead to alteration or contamination of samples. For example, a poor surface seal around the well opening can allow contaminants to move quickly from the land surface into the well water.

Sampling equipment that does not require chlorine disinfection:

If the water level is less than 7 to 10 m (roughly 20 to 30 ft) below land surface, a sample can be collected without contamination and without chlorine disinfection by use of a surface peristaltic or vacuum pump, a sterile vacuum flask, and two lengths of sterile tubing (U.S. Environmental Protection Agency, 1982).

Precautions for collecting samples from monitoring wells:

Purge the well (see NFM 4.2 and 6.0.3.A) while monitoring field measurements, especially measures of turbidity and dissolved oxygen. For wells in which field measurements do not stabilize after increasing the total number of measurements, record the final measurements and proceed with sampling.

Be vigilant in avoiding contamination. The detection of even one coliphage in ground water is cause for concern because it indicates the possible presence of pathogens.

Quality control for ground-water sample collection. Depending on the data-quality requirements of the study, quality-control samples (field blanks and matrix spikes) generally constitute from 5 to 20 percent or more of the total number of samples collected over a given time period. See "Selected Terms and Symbols" in the Conversion Factors section at the end of this chapter, which contains definitions of the quality-control terms shown below in bold type.

- **Field blanks** – Collect field blanks at a frequency of 1 in every 10 to 20 samples if required by data-quality objectives. **Process field blanks before collecting the water sample** by passing sterile DIW through the sampling equipment and into a sterile sample container, and analyzing sterile DIW for fecal indicator viruses and recording results. If no viruses are observed, the use of sterile procedures is confirmed and documented.

  TECHNICAL NOTE: The field blank discussed herein is equivalent to the "pump blank" described in NFM 4.3.1. Refer to NFM 4.3.1 for more information on collecting a field blank for ground-water sampling. A standpipe may be used to collect a field blank, but first must be cleaned and then disinfected. **This type of blank should be collected a week or more ahead of time so that results can be reported before field sampling.**

- **Field replicates** (sequentially collected samples)—Field replicates for ground-water samples are optional and their use depends on study objectives and site conditions. Ground-water samples typically are negative for coliphage.
- **Matrix spikes—**Collect a set of matrix spike samples for each coliphage type when samples are first received from a water source or aquifer type. Once received from a water source or aquifer type, collect a set of matrix spike samples after every 20th sample from that source or type. The matrix spike samples are spiked with known amounts of coliphage by the analyzing laboratory.

SAMPLE PRESERVATION, TRANSPORT, AND HOLDING TIMES 7.2.2.C

After collection, immediately chill samples in an ice chest or refrigerator at 1 to 4°C. **Do not freeze samples.** To ship samples to the laboratory, double bag the sample containers before placing them into the bagged ice in the ice chest. Seal the analytical services request form and chain-of-custody form in double plastic bags and tape this to the inside lid of the ice chest being shipped to the laboratory. Check that the sample identification and relevant information for use by the laboratory have been recorded correctly. **The laboratory must begin the analysis of samples within 48 hours of sample collection.**

The holding time for fecal indicator virus samples is 48 hours from the time of sample collection.

7.2.3 LABORATORY METHODS

Two methods described in this manual for the detection of fecal indicator viruses are the single-agar layer (SAL) method and the two-step enrichment method. The host bacteria recommended for use by these methods are *E. coli* CN-13 for the detection of somatic coliphage and *E. coli* F-amp for the detection of F-specific coliphage. Analytical protocols are available in more detail from the USGS Ohio District Microbiology Laboratory (U.S. Geological Survey, website: http://oh.water.usgs.gov/micro/lab.html#am) (accessed March 31, 2003).

7.2.3.A SINGLE-AGAR LAYER METHOD

The SAL method detects and enumerates somatic and F-specific coliphages in water. It is a plaque assay method that is recommended for use with surface-water samples.

USEPA Method 1602 (U.S. Environmental Protection Agency, 2001b) is a SAL method that requires the addition of host bacteria, magnesium chloride, appropriate antibiotics, and double-strength molten agar to the sample, followed by pouring the total volume of the mixture into plates. After an overnight incubation, the plates from a sample are examined for plaque formation (zones of bacterial host lawn clearing). The plaques are counted and summed for all plates from a single sample. The quantity of coliphage in a sample is expressed as plaques per 100 milliliters. This method requires one overnight incubation; therefore, results are available 24 hours after the beginning of the analysis.

Quality Control. Each laboratory and analyst that uses USEPA Method 1602 must fulfill the following general quality-control requirements, as described in the method:

- **Initial Precision and Recovery (IPR)**—The laboratory and analyst must demonstrate the ability to generate acceptable results by performing an IPR test before analyzing any environmental samples.
- **Method Blanks**—The laboratory must analyze reagent water samples containing no coliphage to demonstrate freedom from contamination. Method blanks should be run with each batch of samples. A batch is defined as all samples analyzed during a single day, up to a maximum of 20 samples per coliphage type.
- **Ongoing Precision and Recovery (OPR)**—The laboratory must demonstrate that the method is in control on an ongoing basis through analysis of OPR samples. OPR samples are reagent-water samples spiked with known amounts of coliphage and analyzed exactly like environmental samples. The laboratory must analyze one OPR sample for each batch of samples. A batch is defined as all samples analyzed during a single day, up to a maximum of 20 samples per coliphage type. The OPR serves as the positive control for Method 1602.

TWO-STEP ENRICHMENT METHOD 7.2.3.B

The two-step enrichment method determines the presence or absence of somatic and F-specific coliphages in water. **This method is recommended for use with ground-water samples.**

USEPA Method 1601 (U.S. Environmental Protection Agency, 2001a) is a two-step enrichment method that requires the enrichment of coliphage in tryptic soy broth supplemented with magnesium chloride, appropriate antibiotics, and host bacteria. After an overnight incubation, samples are spotted onto a lawn of host bacteria specific for each type of coliphage. The spot plates are incubated and examined for lysis zone formation in the lawn. Lysis zone formation indicates the presence of coliphages in the sample. This method requires two overnight incubations; therefore, results are available 48 hours after the beginning of the analysis.

Quality control. Each laboratory and analyst that uses Method 1601 must fulfill the following general quality-control requirements as described in the method.

- **Initial Demonstration of Capability (IDC)**—The laboratory and analyst must demonstrate the ability to generate acceptable results by performing an IDC test before analyzing any environmental samples.
- **Method Blanks**—The laboratory must analyze reagent-water samples containing no coliphage to demonstrate freedom from contamination. The laboratory must analyze one method blank per spot plate.
- **Positive Controls**—The laboratory must analyze positive control samples (reagent water spiked with a known amount of coliphage) to demonstrate that method reagents are performing properly. The laboratory must analyze one positive control per spot plate.
- **Ongoing Demonstration of Capability (ODC)**—The laboratory must demonstrate that the method is in control on an ongoing basis through analysis of ODC samples. The laboratory must analyze one set of ODC samples after every 20 field and matrix spike samples. For each coliphage type, at a minimum, one out of three reagent-water samples spiked with a known amount of coliphage must be positive.

CALCULATION AND REPORTING OF FECAL INDICATOR VIRUSES 7.2.4

The calculation and reporting protocols differ, depending on the laboratory method used. A list of parameter codes for reporting coliphages in the USGS National Water Information System (NWIS) are given in Appendix A7-A, table 3.

- **SAL method** – Count the total number of plaques from all plates for a sample. If the plaques are not discrete, results should be recorded as "too numerous to count" (TNTC), and the remaining sample should be diluted and reanalyzed if possible within 48 hours of collection. Record the result as the total number of plaques per 100 milliliters (plaques/100 mL).
- **Two-step enrichment method** – Record results as presence (1) or absence (2) of coliphage.

For each sample analyzed, document

- **the type of coliphage analyzed,**
- **the bacterial host strain used,**
- **the sample volume analyzed, and**
- **the corresponding QC results from the laboratory.**